

- 58 -

Claims:

1. A virus-like particle (VLP) comprising a fusion polypeptide comprising a polypeptide of interest (POI) and at least a particle-associating portion of a large envelope polypeptide (L).
2. A virus-like particle (VLP) comprising i) a fusion polypeptide comprising a polypeptide of interest (POI) and a particle-associating portion of a large envelope polypeptide (L) and ii) a small envelope (S) polypeptide.
3. The VLP of claim 1 or 2 comprising a fusion polypeptide comprising a polypeptide of interest (POI) and a particle-associating portion of a large envelope polypeptide (L) of an avian hepadnavirus such as duck hepatitis B virus (DHBV) or a functional derivative or homolog thereof.
4. The VLP of claim 2 or 3 comprising a fusion polypeptide comprising a polypeptide of interest (POI) and a particle-associating portion of a large envelope polypeptide (L) of an avian hepadnavirus such as duck hepatitis B virus (DHBV) or a functional derivative or homolog thereof and ii) a small envelope (S) polypeptide of an avian hepadnavirus such as DHBV or a functional derivative or homolog thereof.
5. The VLP of claim 1 or 2 wherein the particle-associating portion of L comprises at least the S domain of L or the S domain of L minus the TM1 domain or a functional derivative or homolog thereof.
6. The VLP of claim 1 or 2 wherein the POI is located in the pre-S domain of L or at the amino terminal side of the S domain of L, or the S domain minus the TM1 domain of L.
7. The VLP of claim 1 or 2 wherein the L polypeptide comprises an amino acid sequence substantially as set forth in SEQ ID NO: 7 or SEQ ID NO: 9 or a

- 59 -

functional derivative thereof or comprises an amino acid sequence having at least 50% similarity to SEQ ID NO: 7 or SEQ ID NO: 9.

8. The VLP of claim 1 or 2 wherein the particle-associating portion of L polypeptide comprises an amino acid sequence substantially as set forth in SEQ ID NO: 7 or SEQ ID NO: 9 or a functional derivative thereof comprising an amino acid sequence having at least 50% similarity to SEQ ID NO: 7 or SEQ ID NO: 9.
9. The VLP of claim 1 or 2 wherein the particle-associating portion of L-polypeptide comprises or consists essentially of amino acids 24 to 107 of SEQ ID NO: 9 or an amino acid sequence having at least 50% similarity thereto.
10. The VLP of any one of claims 1 to 9 wherein the L polypeptide is a DHBV L polypeptide or functional derivative thereof.
11. The VLP of claim 1 or 2 wherein said L polypeptide or particle-associating portion thereof is encoded by a sequence of nucleotides substantially as set forth in SEQ ID NO: 6 or SEQ ID NO: 8 or having at least about 50% similarity to SEQ ID NO: 6 or SEQ ID NO: 8 or a contiguous sequence of nucleotides capable of hybridizing to a complementary form of SEQ ID NO: 6 or SEQ ID NO: 8 under hybridisation conditions of medium stringency.
12. The VLP of claim 1 or 2 wherein said L polypeptide further comprises a signal sequence.
13. An isolated or recombinant polypeptide for use in the assembly of a VLP, comprising a polypeptide of interest (POI) and at least a particle-associating portion of a large envelope polypeptide (L) of an avian hepadnavirus such as DHBV or a functional derivative or homolog thereof.
14. A recombinant polypeptide capable of assembling into a VLP when expressed in a

- 60 -

cell comprising a polypeptide of interest (POI) and at least a particle-associating portion of a large envelope polypeptide (L) of an avian hepadnavirus such as DHBV or a functional derivative of homolog thereof.

15. The polypeptide of claim 13 or 14 wherein the particle-associating portion of L comprises at least the S domain of L or the S domain of L minus the TM1 domain or a functional derivative thereof.
16. The polypeptide of claim 13 or 14 wherein the POI is located in the pre-S domain of L or at the amino terminal side of the S domain of L, or the S domain minus the TM1 domain of L.
17. The recombinant or isolated polypeptide of claim 13 or 14 wherein the L polypeptide comprises an amino acid sequence substantially as set forth in SEQ ID NO: 7 or SEQ ID NO: 9 or a functional derivative thereof or comprises an amino acid sequence having at least 50% similarity to SEQ ID NO: 7 or SEQ ID NO: 9.
18. The recombinant or isolated polypeptide of claim 13 or 14 wherein the particle-associating portion of L polypeptide consists of an amino acid sequence substantially as set forth in SEQ ID NO: 7 or SEQ ID NO: 9 or a functional derivative thereof comprising an amino acid sequence having at least 50% similarity to SEQ ID NO: 7 or SEQ ID NO: 9.
19. The recombinant or isolated polypeptide of claim 13 or 14 wherein the particle-associating portion of L-polypeptide comprises or consists essentially of amino acids 24 to 107 of SEQ ID NO: 9 or an amino acid sequence having at least 50% similarity thereto.
20. The recombinant or isolated polypeptide of claim 13 or 14 wherein said L polypeptide or particle-associating portion thereof is encoded by a sequence of nucleotides substantially as set forth in SEQ ID NO: 6 or SEQ ID NO: 8 or having

- 61 -

at least about 50% similarity to SEQ ID NO: 6 or SEQ ID NO: 8 or a contiguous sequence of nucleotides capable of hybridizing to a complementary form of SEQ ID NO: 6 or SEQ ID NO: 8 under hybridisation conditions of medium stringency.

21. The recombinant or isolated polypeptide according to claim 13 or 14 wherein said L polypeptide further comprises a signal sequence.
22. The recombinant or isolated polypeptide of claim 13 or 14 wherein the L polypeptide is a DHBV L polypeptide or functional derivative thereof.
23. A recombinant nucleic acid molecule for use in making a VLP, said nucleic acid molecule comprising a contiguous sequence of nucleotides encoding a polypeptide of interest (POI) and at least a particle-associating portion of an L polypeptide of an avian hepadnavirus such as DHBV or a functional derivative or homolog thereof.
24. A recombinant nucleic acid molecule for use in making a VLP, said nucleic acid molecule comprising a contiguous sequence of nucleotides encoding i) a fusion polypeptide comprising a polypeptide of interest (POI) and at least a particle-associating portion of an L polypeptide of an avian hepadnavirus such as DHBV or a functional derivative or homolog thereof, and ii) a small envelope (S) polypeptide of an avian hepadnavirus such as DHBV or a functional derivative or homolog thereof.
25. The nucleic acid molecule of claim 23 or 24 wherein the particle-associating portion of L comprises at least the S domain of L or the S domain of L minus the TM1 domain or a functional derivative thereof.
26. The nucleic acid molecule of claim 23 or 24 wherein the POI is located in the pre-S domain of L or at the amino terminal side of the S domain of L, or the S domain minus the TM1 domain of L.

- 62 -

27. The nucleic acid molecule of claim 23 or 24 which encodes an L polypeptide comprising an amino acid sequence substantially as set forth in SEQ ID NO: 7 or SEQ ID NO: 9 or comprises an amino acid sequence having at least 50% similarity to SEQ ID NO: 7 or SEQ ID NO: 9.
28. The nucleic acid molecule of claim 27 which encodes a particle associating portion of L polypeptide consisting essentially of amino acids 24 to 167 of SEQ ID NO: 9 or an amino acid sequence having at least 50% similarity to SEQ ID NO: 9.
29. The nucleic acid molecule of claim 23 or 24 wherein said L polypeptide or particle associating portion thereof is encoded by a sequence of nucleotides substantially as set forth in SEQ ID NO: 6 or SEQ ID NO: 8 or having at least about 50% similarity to SEQ ID NO: 6 or SEQ ID NO: 8 or a contiguous sequence of nucleotides capable of hybridizing to a complementary form of SEQ ID NO: 6 or SEQ ID NO: 8 under hybridisation conditions of medium stringency.
30. The nucleic acid molecule of claim 23 or 24 wherein the L polypeptide further comprises a signal sequence.
31. The nucleic acid molecule of claim 23 or 24 wherein the L polypeptide is a DHBV L polypeptide or functional derivative thereof.
32. A recombinant nucleic acid molecule for use in making a VLP, said nucleic acid molecule encoding a particle-associating portion of an L polypeptide of an avian hepadnavirus such as DHBV or a functional derivative or homolog thereof and comprising one or more cloning sites suitable for accepting a nucleic acid molecule encoding a polypeptide of interest (POI), wherein said POI is expressed together with said L polypeptide.
33. A recombinant nucleic acid molecule for use in making a VLP, said nucleic acid molecule encoding at least a particle-associating portion of an L polypeptide of an

- 63 -

avian hepadnavirus such as DHBV or a functional derivative or homolog thereof, and ii) a small envelope (S) polypeptide of an avian hepadnavirus such as DHBV or a functional derivative or homolog thereof and comprising one or more cloning sites suitable for accepting a nucleic acid molecule encoding a polypeptide of interest (POI), wherein said POI is expressed together with said L polypeptide.

34. The nucleic acid molecule of claim 32 or 33 wherein the particle-associating portion of L comprises at least the S domain of L or the S domain of L minus the TM1 domain or a functional derivative thereof.
35. The nucleic acid molecule of claim 32 or 33 wherein the POI is located in the pre-S domain of L or at the amino terminal side of the S domain of L, or the S domain minus the TM1 domain of L.
36. The nucleic acid molecule of claim 32 or 33 which encodes an L polypeptide comprising an amino acid sequence substantially as set forth in SEQ ID NO: 7 or SEQ ID NO: 9 or comprises an amino acid sequence having at least 50% similarity to SEQ ID NO: 7 or SEQ ID NO: 9.
37. The nucleic acid molecule of claim 36 which encodes a particle-associating portion of L polypeptide consisting essentially of amino acids 24 to 167 of SEQ ID NO: 9 or an amino acid sequence having at least 50% similarity to SEQ ID NO: 9.
38. The nucleic acid molecule of claim 32 or 33 wherein said L polypeptide or particle-associating portion thereof is encoded by a sequence of nucleotides substantially as set forth in SEQ ID NO: 6 or SEQ ID NO: 8 or having at least about 50% similarity to SEQ ID NO: 6 or SEQ ID NO: 8 or a contiguous sequence of nucleotides capable of hybridizing to a complementary form of SEQ ID NO: 6 or SEQ ID NO: 8 under hybridisation conditions of medium stringency.
39. The nucleic acid molecule of claim 32 or 33 wherein the L polypeptide further

- 64 -

comprises a signal sequence.

40. The nucleic acid molecule of claim 32 or 33 wherein the L polypeptide is a DHBV L polypeptide or functional derivative thereof.
41. An isolated and/or recombinant cell comprising the nucleic acid molecule of any one of claims 23 to 31 or expressing the polypeptide of any one of claims 13 to 22 or the VLP of any one of claims 1 to 12.
42. The cell according to claim 41 wherein said cell is a eukaryotic cell, preferably a yeast, avian or mammalian cell.
43. A method of delivering a POI to a subject or cell comprising expressing the POI in a VLP comprising L polypeptide from an avian hepadnavirus such as DHBV or a functional derivative or homolog thereof such that at least part of the POI is expressed on the surface of the VLP and administering the VLP to a subject or cell.
44. The method of claim 43 wherein the VLP is made in an *in vitro* expression system such as a yeast, avian or mammalian expression system.
45. The method of claim 43 wherein the VLP is made *in vivo* in the cells of a subject after administration of the nucleic acid molecule of any one of claims 23 to 31.
46. A method for making a recombinant VLP said method comprising:
 - i) cloning a nucleic acid molecule encoding a polypeptide of interest into an expression vector comprising a particle-associating portion of an L polypeptide of an avian hepadnavirus such as DHBV or a functional derivative or homolog thereof;
 - ii) introducing the recombinant expression vector of step i) into a suitable cell and maintaining same under conditions which allow protein expression and particle assembly with S polypeptide of an avian hepadnavirus such as

- 65 -

- DHBV or a functional derivative or homolog thereof; and
- iii) recovering said virus-like particles from said cells.